The Dominant Epitope of *Borrelia garinii* Outer Surface Protein C Recognized by Sera from Patients with Neuroborreliosis Has a Surface-Exposed Conserved Structural Motif

MARIANNE J. MATHIESEN,[1] ARNE HOLM,[2] MICHAEL CHRISTIANSEN,[1] JENS BLOM,[3] KLAUS HANSEN,[1] SØREN ØSTERGAARD,[2]† AND MICHAEL THEISEN[1]*

Department of Clinical Biochemistry[1] and Department of Molecular Cell Biology,[3] Statens Serum Institut, and Department of Chemistry, Royal Veterinary and Agricultural University,[2] Copenhagen, Denmark

Epitope mapping of outer surface protein C (OspC) by using sera from patients with neuroborreliosis led to the identification of one single major immunodominant epitope within the C-terminal 10 amino acid residues. Peptide binding studies and alanine replacement scanning of the C-terminal decapeptide, PVVAESPKKP, revealed a critical role for the PKKP sequence and its terminal carboxyl group for the binding of immunoglobulin M (IgM) antibodies from patients with Lyme borreliosis. Electron microscopy of antibody-labeled spirochetes indicated that the C-terminal region is exposed on the surface of the spirochete. Based on homology to proteins of known function, this region most probably adopts a polyproline II-like helix, which is found in surface-exposed structures involved in protein-protein interactions. This structural motif is highly conserved in *Borrelia* species causing Lyme borreliosis and subjected to purifying selection. We suggest that the abundance of the C-terminal region of OspC on the surface of *B. burgdorferi* allows a multimeric high-avidity interaction between the spirochete and surface Igs on B cells. The resulting cross-linking of surface Igs on B cells may induce a T-cell-independent B-cell activation without IgM-to-IgG switching, thus explaining the lack of IgG antibodies to OspC in neuroborreliosis.

Outer surface protein C (OspC) of the Lyme borreliosis agents *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* is the major outer surface lipoprotein expressed during the early stages of disease and one of the primary targets for the human immune response against these spirochetes (47). The corresponding *ospC* gene is present in a single copy on a 26-kb circular plasmid (23, 35). Although the *ospC* gene is present in all *B. burgdorferi* sensu lato isolates, the corresponding gene product is produced in only 40 to 45% of European strains (41, 48) and very rarely in U.S. strains upon in vitro isolation. It is known that *ospC* expression is temperature dependent and may be induced by raising the temperature of the growth medium (36, 38, 47). Accordingly, OspC is not produced by spirochetes in unfed ticks (7) but expression is turned on during the blood meal (36) and in the vertebrate host (28). It has been proposed that OspC is involved in the migration of *B. burgdorferi* from the midgut of the tick to the salivary glands and also in the survival of *B. burgdorferi* in the vertebrate host (36).

OspC homologs have been found in other *Borrelia* species (24). Most notable is the similarity to the variable major lipoprotein, Vmp33, of *Borrelia hermsii* (2). It has been suggested that these OspC homologs are functionally related (2, 24, 25, 40, 41); however, no shared function has yet been proposed for this family of proteins.

Since OspC elicits an early immune response in the human host, several attempts have been made to develop diagnostic assays based on OspC (10, 11, 31, 44, 45) and to examine its vaccine potential (1, 12, 17, 32, 33). The very high variability

encountered in OspC (18, 19, 22, 37, 40, 41) may, however, complicate the further use of this antigen. More knowledge is therefore needed about the epitopes in OspC before OspC can be used rationally in vaccine formulations or in diagnostic assays. In this study, we present the identification and characterization of a single major epitope in the conserved C-terminal region of OspC, defined by immunoglobulin M (IgM) antibodies in sera from patients with neuroborreliosis (NB). We provide data which indicate that the C-terminal region is surface exposed.

MATERIALS AND METHODS

Expression of recombinant OspC fragments. For the structures of the various plasmids used in this study, see Fig. 1. All plasmid constructs were verified by DNA sequencing.

(i) pBF39. The partial *ospC* gene lacking the codons encoding the C-terminal 7 amino acids was amplified from genomic DNA by using standard PCR conditions and the two primers BF22 (5′-ATA GAT ATC AAT AAT TCA GGT GGG GAT TC) (nucleotides 58 to 77, counting from the A of the ATG start codon) and BF23 (5′-TTT GAT ATC TCA CAC AAC AGG ATT TGT AAG CTC TTT AAC) (nucleotides 600 to 574), cut with *Eco*RV (underlined), and ligated into pMST24 digested with *Sma*I. This vector provides the OspC coding sequence with an in-frame start codon and a hexahistidyl tag (42). Recombinant protein OspC$_{19–200}$ was purified by metal ion affinity chromatography on a Ni$^+$ column (42).

(ii) pBF147. The coding region for the full-length OspC protein was amplified from genomic DNA by using standard PCR conditions and the two primers BF22 and BF65 (5′-TTT GAT ATC TCA AGG TTT TTT TGG ACT TTC TGC) (nucleotides 621 to 601). The amplified gene was cut with *Eco*RV (underlined) and subsequently cloned into pMST24 digested with *Sma*I. Recombinant protein OspC$_{19–207}$ was purified as described above.

Peptide synthesis and conjugation. Solid-phase peptide synthesis was performed by multiple-column peptide synthesis (9, 16). All peptides were synthesized with 9-fluorenylmethoxycarbonyl (Fmoc) amino acids (MilliGen and Calbiochem-Novabiochem), using *O*-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and *N*-hydroxy-benzotriazole as coupling agents. An acid-labile H-Pro-2-ClTrt resin (Novabiochem; *s* = 0.8 mmol/g) was used to prepare C-terminal proline-containing peptide carboxylates. Peptide structure and homogeneity were confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a Fisons TofSpec E instrument and by high-pressure liquid chromatography on a C$_{18}$ reversed-phase column (Waters Rad-Pak Delta-Pak C$_{18}$). The concentrations of the

* Corresponding author. Mailing address: Department of Clinical Biochemistry, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. Phone: (45) 32683779. Fax: (45) 32683228. E-mail: mth@ssi.dk.

† Present address: Novo Research Institute, Bagsværd, Denmark.

peptide samples were determined by amino acid analysis performed with the PICOTAG system (Waters). For preparation of conjugates, peptides were synthesized with an additional cysteine residue at the amino terminus and the resulting peptide was added to purified protein derivative (Statens Serum Institut) by means of the heterobifunctional reagent SPDP [*N*-succinimidyl-3-(2-pyridylthio)propionate; Sigma, St. Louis, Mo.] by the method of Granier et al. (29).

Sera. Sera were obtained from 100 consecutive Danish patients with NB. All the patients had been hospitalized in 1994 (58 males and 42 females between 4 and 80 years of age; median age, 49 years). The selection was based primarily on a positive *B. burgdorferi* specific intrathecal antibody synthesis (13). The diagnosis was further based on clinical evidence and on the presence of lymphocytic pleocytosis in cerebrospinal fluid (CSF). The diagnosis was specified by the presence of lymphocytic meningoradiculitis with typical painful radiculitis, mononeuritis multiplex (Bannwarth's syndrome), radiculomyelitis, monosymptomatic facial palsy (all children), subacute lymphocytic meningitis, and chronic progressive encephalomyelitis. Before treatment, all but two of the patients had lymphocytic pleocytosis in CSF, with counts of 5×10^6 to $1,200 \times 10^6$ cells per liter (median cell count, 124×10^6 cells per liter); in two patients, CSF cytology was not examined. Of the 100 patients, 48 recalled a previous encephalomyelitis-like skin lesion. The duration of disease, defined as the time from onset of neurological symptoms until diagnostic blood and CSF samples were taken, ranged from 1 to 26 weeks (median duration, 3 weeks).

The antipeptide antiserum was obtained by priming female BALB/c mice (6 to 8 weeks old) with 10^6 CFU of *Mycobacterium bovis* BCG (Copenhagen strain; Statens Serum Institut) per mouse delivered intraperitoneally in 500 μl of phosphate-buffered saline (PBS) followed by three intraperitoneal injections, at 2-week intevals, of PPD-PVVAESPKKP conjugate adsorbed to alum. The rabbit antiserum raised against purified OspC from DK6 has been described previously (41).

ELISA. In the recombinant enzyme-linked immunosorbent assay (ELISA), microtiter plates (Maxisorb; Nunc, Roskilde, Denmark) were coated overnight at 4°C with $OspC_{19-207}$, or $OspC_{19-200}$ at 0.4 μg/ml and blocked with 3% (wt/vol) milk powder (Matas) in PBS–0.05% (vol/vol) Tween 20.

In the peptide ELISA, microtiter plates were coated overnight at 4°C with streptavidin (Zymed, South San Francisco, Calif.) at 2.5 μg/ml in citrate buffer (pH 5), washed, and then incubated overnight at 4°C with the biotinylated peptide at 0.1 μg/ml in PBS containing 0.37 M NaCl, 0.5% (vol/vol) Tween 20, and 1% (wt/vol) milk powder. Serum was incubated at a 1:200 dilution in PBS containing 0.7 M NaCl, 0.1% (vol/vol) Tween 20, and 1% (wt/vol) milk powder. Antibody binding was detected with peroxidase-conjugated rabbit anti-human IgM (no. P215; Dako, Copenhagen, Denmark). The 98% specific diagnostic cutoff levels were based on results from 100 healthy Danish blood donors and were optical densities of 0.230 for the recombinant ELISA and 0.450 for the peptide ELISA.

Competition ELISA. Serial dilutions of $OspC_{19-207}$, $OspC_{19-200}$, overlapping synthetic peptides covering the C-terminal region, or analogs of this region with alanine substitutions were added to sera diluted 1,000-fold (serum a) or 200-fold (sera b to f) in 1% (wt/vol) milk powder in PBS–0.1% (vol/vol) Tween 20. The mixtures were incubated overnight at 4°C, and the effect on the binding to $OspC_{19-207}$-coated ELISA plates was determined. The six serum samples used were randomly selected among the anti-OspC high-titer sera from NB patients.

Electron microscopy. *B. garinii* DK6 was grown to mid-log phase in BSKII medium, and 10^8 cells were harvested by centrifugation at $3,000 \times g$ for 20 min at room temperature. The cells were washed once by centrifugation at $3,000 \times g$ in Tyrode's buffer (14) and gently resuspended in 1 ml of the same buffer. This treatment has been shown to keep the surface layer intact (14). Formvar-coated, carbon-reinforced 400-mesh copper grids were irradiated for 10 min with UV light and then incubated for 5 min on drops of *B. garinii* DK6 in Tyrode's buffer. The grids were blocked for 30 min in PBS containing 3% (wt/vol) bovine serum albumin (Sigma) and then incubated with 50 μl of antiserum diluted 1:20 in PBS containing 1% (wt/vol) bovine serum albumin and 1% (vol/vol) Tween 20 (PBST). After five 5-min washes in PBST, the grids were incubated in 50 μl of goat anti-mouse Igs conjugated to 10-nm-diameter gold spheres (no. G441; Dako) and diluted 1:30 in PBST and then washed sequentially in PBST and distilled water. The cells were negatively stained with 1% (wt/vol) ammonium molybdate solution (pH 7.4) for 10 s, air dried, and examined by transmission electron microscopy with a Philips 201 C instrument operated at 60 kV.

RESULTS

The C-terminal region contains the major epitope recognized by patient serum antibodies. OspC is composed of conserved N- and C-terminal regions and a variable central region (Fig. 1). To locate the immunodominant regions on OspC, the full-length OspC comprising amino acids 19 to 207 and a truncated OspC comprising amino acids 19 to 200 were used in an IgM ELISA testing 100 sera from patients with active NB. Of these sera, 48 showed a significant antibody reactivity with $OspC_{19-207}$ and only 4 reacted with $OspC_{19-200}$ (Fig. 1A and B). The C-terminal 7 amino acids are thus critical for IgM
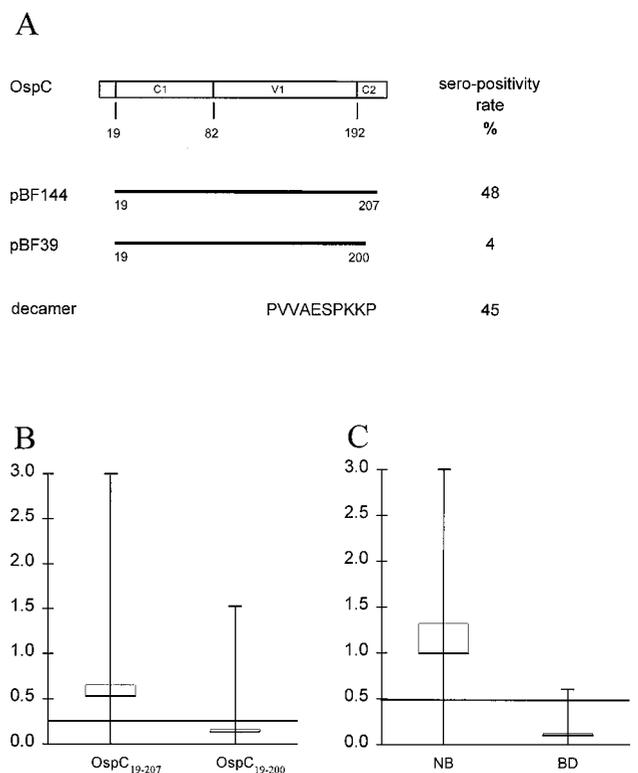

FIG. 1. Identification of the major epitope of OspC. (A) A schematic representation of the two recombinant OspC proteins encoded by plasmids pBF144 and pBF39. Below is indicated the sequence (in single-letter code) of a synthetic peptide comprising the C-terminal 10 amino acids. The open box indicates the amino acid sequence of OspC from *B. garinii* DK6. C1 and C2 indicate relatively conserved regions, and V1 indicate the relatively variable region (22, 40). The NH₂-terminal 19 amino acids constitute the signal peptide. Numbers below the box indicate amino acid residues. To the right is listed the percentage of sera from NB patients with a response greater than the 98% percentile for the 100 Danish blood donors. (B and C) Mean (horizontal bars) and range (vertical bars) of the IgM reactivity against $OspC_{19-207}$ and $OspC_{19-200}$ of the 100 serum samples from NB patients (B) and the anti-PVVAESPKKP IgM reactivity of sera from the 100 NB patients and from Danish blood donors (BD) (C); the thin brackets represent the 95% confidence interval. The 98% specific cutoff levels based on the examination of 100 Danish blood donors were optical densities of 0.230 for anti-$OspC_{19-207}$ and anti-$OspC_{19-200}$ IgM reactivity and 0.450 for anti-peptide IgM reactivity.

binding. This conclusion was confirmed by measuring the antibody reactivity in the 100 serum samples toward a synthetic peptide comprising the C-terminal 10 amino acid residues (Fig. 1A and C). Of these sera, 45 displayed antipeptide reactivity and 42 of the 45 also reacted with $OspC_{19-207}$.

In contrast to the results obtained above, anti-OspC antibodies obtained by immunizing rabbits with purified OspC from DK6 (41) did not display antipeptide reactivity.

Competition experiments. The IgM antibody response to OspC was analyzed in detail in six anti-OspC high-titer serum samples from NB patients by competition experiments.

In the first set of experiments, each of the two recombinant OspC polypeptides were added to the sera and the effect on the binding to $OspC_{19-207}$-coated ELISA plates was determined. Figure 2 shows the results obtained for two representative serum samples. Only the addition of recombinant $OspC_{19-207}$ inhibited the anti-OspC reactivity, while the addition of $OspC_{19-200}$ had no effect on the binding of IgM antibodies to $OspC_{19-207}$ (Fig. 2A).

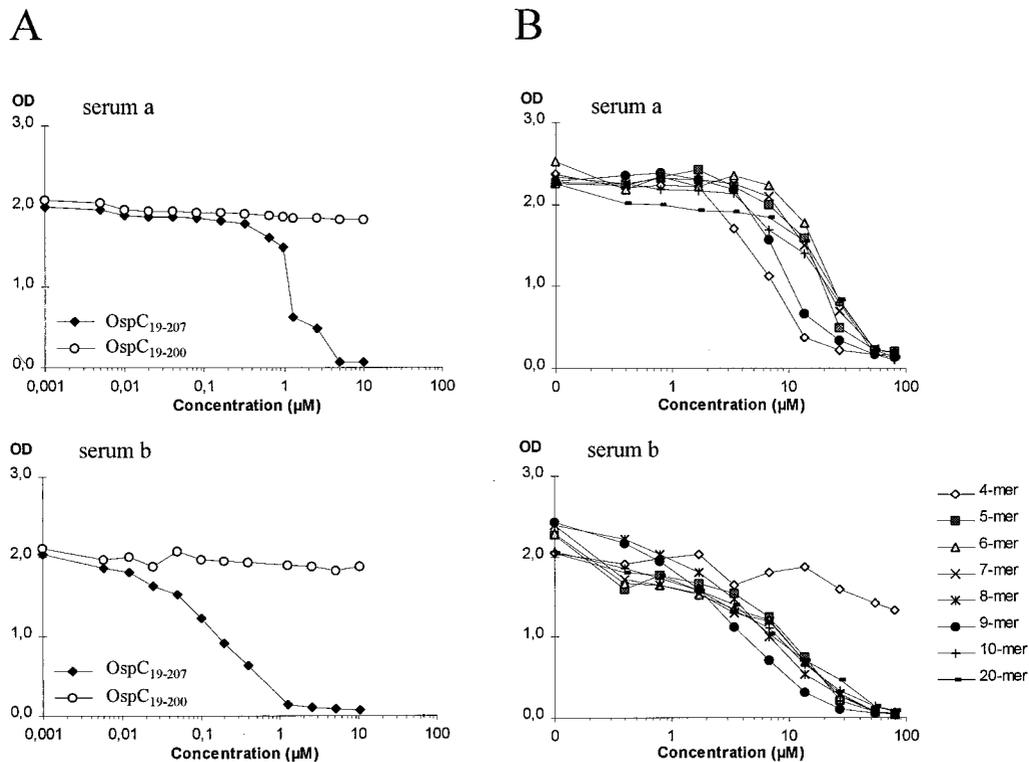In the second set of experiments, synthetic peptides corre-

A

B

FIG. 2. Differential serum IgM recognition of recombinant proteins and overlapping synthetic peptides derived from the C-terminal region. ELISA plates were coated with $OspC_{19-207}$. Shown are the results obtained for two serum samples from patients with NB (serum a and serum b) diluted 200-fold and preincubated with the recombinant proteins $OspC_{19-207}$ (○) and $OspC_{19-200}$ (◆) (A) or with eight overlapping synthetic peptides comprising the C-terminal region (B) at the indicated concentrations before addition to the wells. OD, optical density.

sponding to the C-terminal 4, 5, 6, 7, 8, 9, 10, and 20 amino acids were tested for their ability to inhibit the binding of serum IgM antibodies to $OspC_{19-207}$. The avidity of the sera against the above peptides, compared to the avidity against $OspC_{19-207}$, was generally lower by a factor of 5 to 50 (compare Fig. 2A and B). In four of the six serum samples, the anti-OspC antibody reactivity was inhibited by all peptides; one example is shown in Fig. 2B (serum a). The anti-OspC antibody reactivity in serum b (Fig. 2B) was only slightly affected by the addition of the 4-mer but was inhibited by the 5-mer and longer peptides. The last serum sample, however, was unaffected by the addition of any of the peptides, even though it also displayed antipeptide reactivity in the peptide ELISA (data not shown). In conclusion, the major antigenic determinant recognized by anti-OspC antibodies in five of the six sera is associated with the C-terminal 5 amino acids.

Fine specificity of anti-OspC IgM antibodies in patient sera. To identify amino acids that are important for binding to anti-OspC antibodies, a series of peptides were synthesized in which each of the 9 nonalanine residues in the C-terminal region was sequentially replaced with alanine; in all cases the remaining nine residues were maintained, and in the case of the single alanine residue (residue 201), it was replaced with phenylalanine. These peptides were tested for their ability to compete with $OspC_{19-207}$ for the binding of IgM antibodies in the five serum samples (serum a to serum e) from NB patients which were all inhibited by the decamer (Fig. 3). An alanine substitution at position 207 (Pro-207) or replacement of the carboxyl group with an amine group greatly reduced the ability of the peptides to compete with $OspC_{19-207}$ for the binding of IgM antibodies in all five serum samples (Fig. 3). Substitutions at positions 204, 205, and 206 (Pro-204, Lys-205, and Lys-206)

resulted in peptides that were impaired in their ability to compete with $OspC_{19-207}$ in four serum samples (b, c, d, and e), two serum samples (a and c), and four serum samples (b, c, d, and e), respectively. Alanine substitutions at residues 198, 199, 200, 202, and 203 and the phenylalanine substitution at residue 201 had no effect on the binding of serum antibodies in any of the five serum samples (Fig. 3). Thus, the C-terminal 4 amino acids, as well as the terminal carboxyl group, play a critical role in the binding of IgM antibodies to OspC.

Sequence variation in the C-terminal region of OspC. Sequence variation in the C-terminal conserved region was analyzed by retrieving the nucleotide sequences of *ospC* of 23 *B. garinii* isolates from the EMBL database. The deduced amino acid sequences of the C-terminal region were compared with that of OspC from DK6. In 11 of the strains, the C-terminal 10 amino acid residues were identical to those in the DK6 sequence. Ten strains contained a single amino acid substitution and two isolates contained two substitutions in this region. The locations of the substitutions are shown in Fig. 4. All amino acid substitutions except the lysine-to-asparagine substitution at position 206 correspond to evolutionarily conserved replacements. The proportion of nonsynonymous (amino acid-altering) nucleotide substitutions per nonsynonymous site (p_N) and the proportion of synonymous nucleotide substitutions per synonymous site (p_S) in the C-terminal region were calculated. If this region is subjected to purifying selection, meaning that there is a selection against nonsynonymous substitutions due to functional constraints, p_S is expected to exceed p_N. The mean values for p_S and p_N and the standard error of the mean calculated by the method of Nei and Gojobori (30) were 0.1510 ± 0.0155 and 0.0434 ± 0.0299, respectively. Thus, p_S is significantly larger than p_N ($P < 0.01$, Student's *t* test),
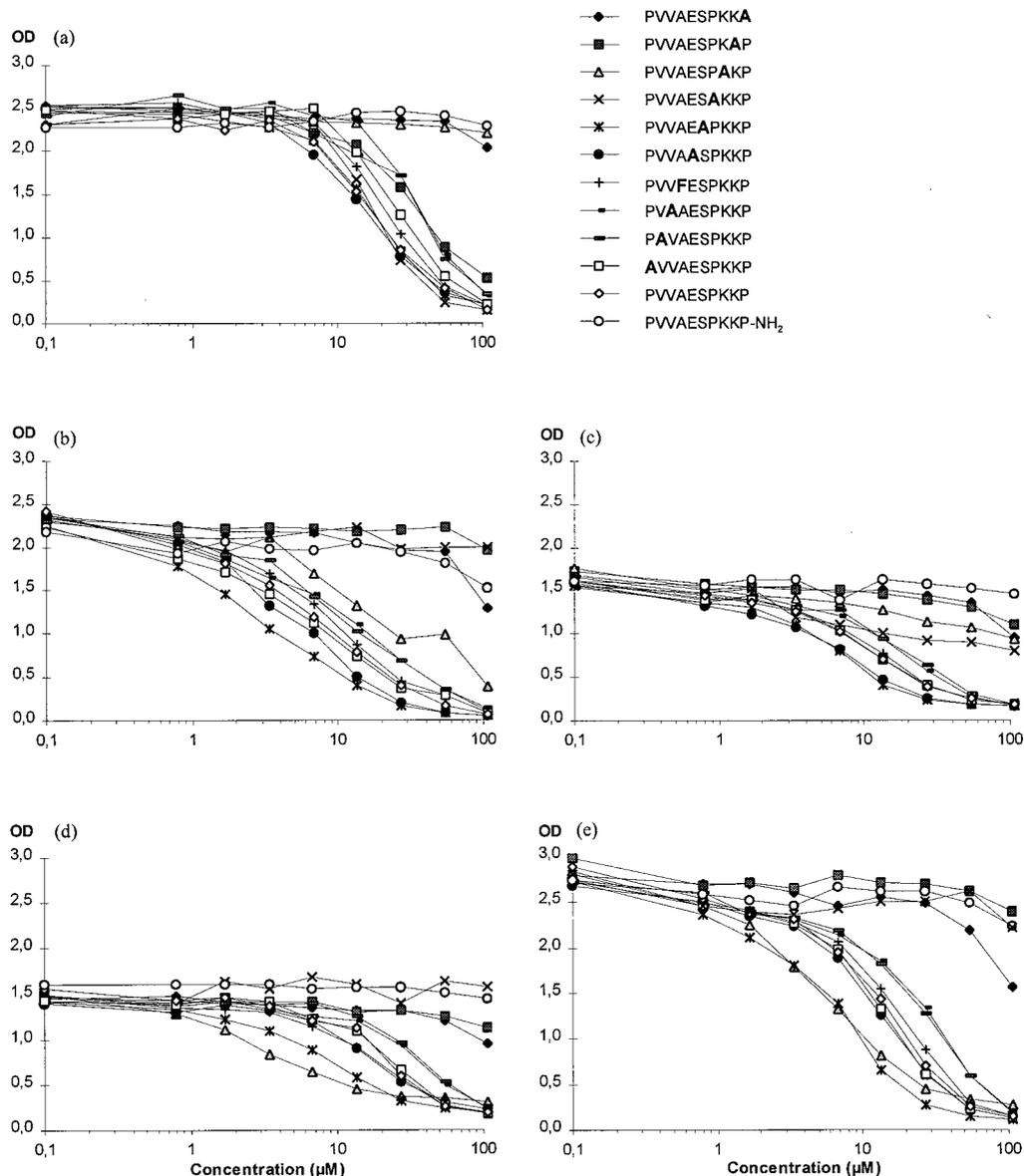
FIG. 3. Fine specificity of OspC-specific IgM antibodies in patient sera. Serum samples from five patients with NB were diluted 1,000-fold (serum a) or 200-fold (sera b to e) and preincubated with the peptides PVVAESPKKP-CONH$_2$, PVVAESPKKP-CO$_2$H, or alanine-substituted analogs for each residue at the indicated concentrations before addition to wells coated with OspC$_{19-207}$. OD, optical density.

demonstrating that this region of OspC from *B. garinii* is under purifying selection. A similar analysis performed on *ospC* sequences obtained from *B. burgdorferi* sensu stricto and *B. afzelii* strains showed that this region of OspC is also conserved in these species.

Localization of the C terminus of OspC by electron microscopy. Immunofluorescence studies with antisera against recombinant OspC have led to the conclusion that the majority of OspC is not surface exposed on cultured spirochetes (1, 4). To determine whether the C-terminal region is accessible for interaction with antibodies, we have raised an antiserum against a synthetic peptide corresponding to the C-terminal 10 amino acid residues of OspC. In a Western blot analysis of a DK6 cell lysate, this antiserum recognized a single band corresponding to OspC (data not shown). When this antiserum was used as a probe, intense labeling was detected on the
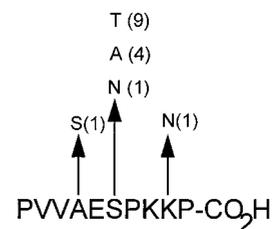
FIG. 4. Amino acid polymorphism in the C-terminal epitope. The deduced amino acid sequence of OspC from DK6 is shown. Polymorphic positions and the amino acid substitutions are indicated. The number in parentheses next to a substitution represents the number of substitutions among OspC alleles from 23 *B. garinii* isolates.
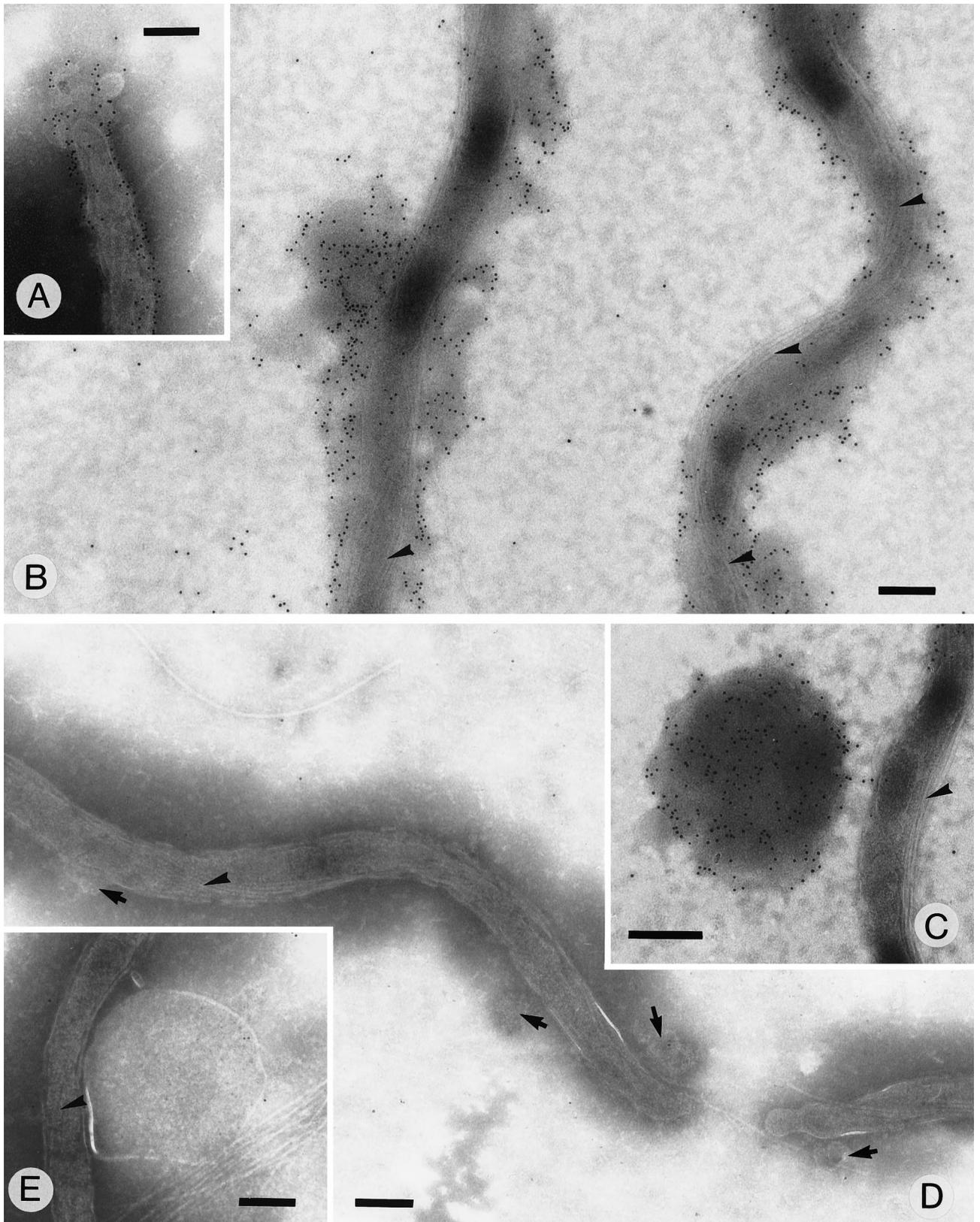
FIG. 5. Immunoelectron micrograph of unfixed cells. *B. garinii* DK6 cells negatively stained with 1% ammonium molybdate are shown. The cells were labeled with a mouse anti-PVVAESPKKP antiserum (A to C) or with the preimmune serum (D and E). Localization of mouse antibodies was detected with goat anti-mouse IgG conjugated to 10-nm-diameter gold beads. (A and B) The surface layer on intact organisms is seen decorated with gold particles. (C) A labelled flake of the surface layer is seen detached from the organism. (D) Intact organisms are seen with unlabelled surface layers (arrows). (E) A large unlabelled flake is shown. Bands of endoflagella are marked by arrowheads. Bars, 0.2 pm.

surface of unfixed *B. garinii* DK6 cells (Fig. 5A and B). The labeling was associated with the outermost layer surrounding intact cells. Labeling was also associated with flakes of detached S-layer material (Fig. 5C). This layer appears similar to the surface layer described previously (14). No labelling was detected when the preimmune serum was used as the probe (Fig. 5D and E). Thus, the C-terminal region of OspC on cultured spirochetes is accessible for interaction with Igs.

DISCUSSION

Epitope mapping studies with sera from patients with NB identified a single major epitope of OspC located in the C-terminal region. The amino acid sequence comprising this epitope is highly conserved among *Borrelia* species causing Lyme borreliosis and has not been identified in other *B. burgdorferi* proteins. The C-terminal decapeptide contains the structural motif PXXP, found in polyproline II-like helices. We have shown that the C-terminal region of OspC, in particular the prolines in residues 198, 204, and 207 involved in the PXXP motif, is subjected to purifying selection, suggesting a functional constraint on this region of OspC.

Although OspC is located in the outer membrane of *B. burgdorferi* (46), several studies have indicated that this protein has a limited surface exposure on cultured spirochetes (1, 4). The results presented here indicate that the C-terminal region of OspC decorates the surface of unfixed *B. garinii* DK6 cells.

The C-terminal heptapeptide in OspC is immunodominant, since the majority of sera from patients with NB react with OspC$_{19-207}$ but not with OspC$_{19-200}$. Furthermore, the analysis of specificity revealed that in five of six serum samples, the prolines in residue 204 and 207 are critical to binding. The immunological reactivity thus seems to depend on residues critical for the maintenance of a polyproline II-like helical structure.

We conclude that the C terminus of OspC is surface exposed and immunodominant and that the purifying selection of the PXXP motif reflects that the C terminus adopts an elongated polyproline II helix of importance for the spirochete. We propose that in the vertebrate host, the surface of *B. burgdorferi* is a repetitive structure with the C-terminal region of OspC as the basic unit.

A polyproline II helix is the preferred conformation of proline-rich regions characterized by a rigid extended structure with $\varphi = -78°$ and $\psi = +146°$ (3). Due to the restriction on these angles, the polyproline II-like helix can be considered a "sticky arm" which binds rapidly and reversibly to other proteins (for a review, see reference 43). The polyproline II helix has been identified as a common structural element of various proteins engaged in host-parasite interactions (21, 34, 49) or in signal transduction (5, 39). For example, alanine replacement of proline residues in the hypothetical polyproline II-like helix of neuropeptide Y showed that this structure is involved in both potency and affinity to central nervous system receptors (8).

The C-terminal region of the OspC homolog, Vmp33, from the relapsing-fever agent *B. hermsii* contains the PXXP motif repeated four times (2). Relapsing fever, like Lyme borreliosis, may involve the nervous system; however, it is not yet known whether the C-terminal regions of OspC and Vmp33 play the same role.

Our hypothesis, i.e., that the surface of *B. burgdorferi* is covered by a repetitive unit composed of the C-terminal region of OspC (assuming an extended polyproline II helical structure), may explain why the humoral immune response against OspC in NB is primarily of the IgM type and why very few patients have IgG antibodies against OspC (26, 45, 47). An ordered display of multiple identical epitopes is thought to

induce T-cell-independent activation of B cells by cross-linking surface Igs (for a review, see reference 27). Thus, immunization with synthetic amino acid polymers (9, 15) or with live *Escherichia coli* expressing multiple copies of a recombinant epitope on the cell surface (20) stimulates T-cell-independent antibody responses. However, when this latter epitope was expressed as part of a periplasmic protein, the response became T-cell dependent (20). T-cell-independent humoral responses (non-major histocompatibility complex restricted) are considered less efficient than the major histocompatibility complex-restricted responses due to the absence of Ig switching, affinity maturation, and memory formation. Thus, the antibody response elicited by a repetitive structure would be expected to be of the IgM type, of low avidity toward the organism, and nonneutralizing. Indeed, as shown in this report, IgM antibodies against OspC in NB sera are of low affinity, and it is characteristic of the Lyme borreliosis infection that IgG antibodies against OspC are rare (26, 45, 47). Moreover, CD40L-deficient mice infected with *B. burgdorferi* are capable of eliciting an antibody response against OspC which is T-cell independent (6). The surface expression of OspC in the vertebrate host may thus suppress the development of protective immunity against *B. burgdorferi* by inducing a nonneutralizing IgM response without memory formation. Our suggested mechanism may be the driving principle behind the purifying selection of the C-terminal region of OspC and, in particular, the proline residues critical for the maintenance of a polyproline II helical structure.

Due to the surface exposure and immunological availability, the C-terminal region of OspC would seem to be a good vaccine candidate. However, two observations indicate that it may be difficult to induce IgG antibodies against this region. First, few Lyme borreliosis sera have IgG antibodies against OspC (26, 45, 47), and second, rabbits immunized with gel-purified native OspC in Freund's complete adjuvant do not produce antibodies to the C-terminal region. This latter observation supports our hypothesis that the spatial organization of OspC in the outer membrane is decisive for the development of naturally occurring antibodies. Thus, to obtain a high-titer, long-lasting immune response, it may prove necessary to couple the C-terminal region of OspC to a strong T-cell epitope, e.g., purified protein derivative.

In conclusion, we have shown that the C-terminal region of OspC is surface exposed and immunodominant and that a PXXP motif contained in the sequence is conserved within Lyme borreliosis spirochetes and is subjected to purifying selection. Moreover, the residues important to the PXXP motif are critical for the maintenance of the immunological reactivity of the C terminus of OspC. Finally, we offer a novel hypothesis to explain how *B. burgdorferi* evades host immunity by presenting an epitope that induces T-cell independent B-cell activation, leading to a failure in affinity maturation and memory formation.

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